NOTE TO FILE
BNF40
March 11, 1997

Subject: Insect Protected Corn Line DBT418

Keywords: corn, *zea mays*, lepidopteran insect resistant, *Bacillus thuringiensis* subsp. *kurstaki cryIA(c)* gene, insect control protein CryIA(c), European corn borer, herbicide resistance, *bar* gene, phosphinothricin acetyltransferase (PAT) protein, glufosinate, partial protease inhibitor II (*pinII*) gene from potato, *bla* gene

Background

In a submission dated September 30, 1996, DEKALB Genetics Corporation provided summary information to support their safety and nutritional assessment of their insect resistant corn line containing transformation event DBT418. Additional information to address potential safety concerns regarding the presence of the ampicillin resistance marker (β-lactamase) gene in their transgenic corn was submitted by DEKALB on November 25, 1996.

Intended Effect and Food/Feed Use

The intended technical effect of this genetic modification of corn is to confer resistance to lepidopteran insects, including the European corn borer. DEKALB Genetics Corporation field tested the insect reisistant corn plants in 1993-1995 and observed resistance to European corn borer. DEKALB used the *cryIA(c)* gene from *Bacillus thuringiensis* subsp. *kurstaki* to confer insect resistance in the DBT418 corn line. As a selectable marker, the *bar* gene originally isolated from *Streptomyces hygroscopicus* which encodes phosphinothricin acetyltransferase (PAT) was used in production of the transgenic corn line DBT418. PAT confers resistance to the herbicide phosphinothricin, also known as glufosinate. DEKALB states that the PAT protein in DBT418 is identical to the PAT protein in DEKALB's glufosinate resistant maize line DLL25 for which DEKALB previously consulted FDA.

Corn is one of the world's primary cereal grain crops. Corn grain (kernels) has both animal feed and human food applications. Human food use of the grain includes direct consumption of the kernels and the production of high fructose corn syrup, glucose, corn oil, starch, ethanol and corn meal. Greater than 80% of corn is used as animal feed, primarily for poultry, swine, cattle, and dairy cows and constitutes a major component of the diet of such animals. Both corn grain and silage are used as animal feed. Animal feed uses for the grain include the kernel itself, which may be fed whole or in a processed form, and byproducts from the production of human food, such as hominy feed or corn steep liquor. The entire plant may also be fed as green chop or in a fermented state as silage.

Molecular Alterations and Characterization

DEKALB introduced three plasmids simultaneously into embryogenic corn cells (an inbred line designated AT824) using microprojectile bombardment. The basic constituents of these plasmids are as follows:

(1) The insect resistance gene, *cryIA(c)*, under control of a chimeric OCS-35S promoter which is a strong promoter derived from the Cauliflower Mosaic Virus (CaMV). The *cryIA(c)* gene used encodes the first 613 amino acids of the naturally occurring *Bacillus thuringiensis* subsp. *kurstaki* CryIA(c) protein. The *cryIA(c)* gene was modified to contain an increased number of codons that are preferred for expression in maize.

(2) The *bar* gene, which encodes the PAT protein and confers herbicide resistance, under control of the 35S-CaMV promoter.

(3) The protease inhibitor II gene from potato (*pinII*) under control of the 35S-CaMV promoter in combination with the first intron from the corn *alcohol dehydrogenase-1* (*adh1*) gene which is an enhancer of gene expression.

DEKALB performed Southern blot analysis to determine the presence or absence of an inserted gene in the transgenic plant and the number of copies of the transgene. The results presented in their safety assessment indicate that, in DBT418 plants, there are approximately two copies of the *cryIA(c)* gene, two copies of the *bar* gene (one intact and one rearranged), and one incomplete copy of the potato *pinII* gene. These genes were introduced on plasmids that contained the *bla* gene and the ColE1 origin of replication. Approximately four intact copies and one partial copy of the *bla* gene and four copies of the ColE1 origin of replication were detected in DBT418 DNA.

DEKALB addressed the potential for transfer of the ampicillin resistance marker gene to microorganisms in animal guts. DEKALB stated a number of reasons why they felt horizontal transfer of the ampicillin resistance gene would be prevented from occurring. In addition, DEKALB conducted experiments to estimate the maximum potential frequency of gene transfer from maize DNA to gut microflora. These experiments took into account a variety of factors that would be necessary for DNA to be successfully transferred to, and incorporated into the DNA of gut microflora. The experiments were designed using *E. coli* to overestimate the frequency of transformation. DEKALB's experiments led them to conclude that an expected frequency of DNA transfer from maize DNA to gut microflora would be less than 2×10^{-27}. From this information, DEKALB claimed that the likelihood of spontaneous transformation of rumen microflora with DNA derived from partially digested plant tissue is extremely remote, and therefore, DEKALB concluded that there are no safety concerns related to the presence of the ampicillin resistance marker gene in their transgenic corn.

DEKALB tested for protein levels in three genotypes that contained the DBT418 transformation event. DEKALB found that CryIA(c) and PAT proteins were expressed in the leaf, root, kernel, and whole plants and that PIN II protein and β-lactamase were not expressed in the transgenic corn (shown by Western blot analysis). According to DEKALB, none of the genetic elements introduced to produce DBT418 corn are known to produce any toxins or allergens.

Expressed Proteins and Regulatory Considerations

According to DEKALB, the inserted DNA is capable of expressing two proteins, CryIA(c) and PAT. DEKALB reports that the CryIA(c) protein is expressed at about 0.0001% of total protein in grain and a maximum of about 0.0002% of total protein in whole plants. Maximum levels of the PAT protein were about 0.0175% of total protein in grain and about 0.139% of total protein in whole plants. DEKALB concludes that the CryIA(c) and PAT proteins will not be macroconstituents in the human or animal diet. The safe use of insecticidal proteins as pesticides and the use of selectable markers as pesticidal inert ingredients in the development of insect resistant plant varieties are regulated by the Environmental Protection Agency (EPA) under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA). Therefore, we have not addressed the safe use of CryIA(c) as a pesticide or the safe use of PAT as a pesticidal inert ingredient.

Nutritional Assessment

The intent of the genetic modifications made by DEKALB was to produce insect resistant corn. DEKALB did not anticipate any other effect. Nonetheless, to preclude any possibility that an unintended effect may have rendered corn inferior for food or feed consumption, DEKALB conducted analysis on corn grain and forage from several field trials and compared the nutrient composition of their transgenic corn line DBT418 to that of a nontransgenic control. DEKALB analyzed for protein, oil, fiber, ash, moisture, and starch. The composition of DBT418 grain and forage for the new corn variety was determined to be not substantially different from that of grain or forage from nontransgenic control plants of the same hybrid. DEKALB also analyzed the amino acid composition of grain harvested from the DBT418 hybrid and control plants and found no significant differences.

Conclusions

Based on the safety and nutritional assessment DEKALB has conducted, DEKALB has concluded, in essence, that the new corn variety (DBT418) that they have developed is not materially different in any respect relevant to food or animal feed safety from corn varieties currently on the market and that it does not raise issues that would require premarket review or approval by FDA. At this time, based on DEKALB's description of its data and analysis, the agency considers DEKALB's consultation on their DBT418 corn line to be complete.

Wendy J. Dixon, Ph.D.